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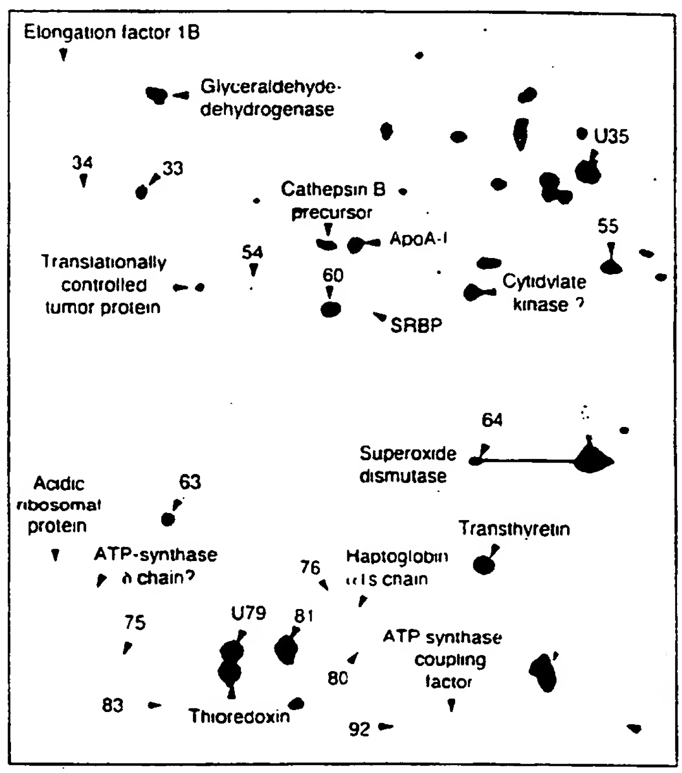
(54) Title: A METHOD FOR DETECTING GROWING CELLS USING TRANSLATIONALLY CONTROLLED TUMOR PROTEIN p21

(57) Abstract

This invention relates to a novel method for detecting growing cells including cancer cells based upon elevated expression of a gene designated Translationally Controlled Tumor Protein p21. The invention provides for direct and indirect methods of quantifying this marker including immunoassays and nucleic acid hybridization assays.



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A METHOD FOR DETECTING GROWING CELLS USING TRANSLATIONALLY CONTROLLED TUMOR PROTEIN p21

BACKGROUND OF THE INVENTION

This invention relates to a novel method for detecting growing cells including cancer cells based upon elevated expression of a gene designated translationally controlled tumor protein p21. The invention provides for direct and indirect methods of quantifying this marker including immunoassays and nucleic acid hybridization assays.

SUMMARY OF THE INVENTION

This invention provides for methods of detecting a cancerous condition in a human comprising: (a) removing a clinical sample from the human; (b) detecting the level of translationally controlled tumor protein p21 (TCTPp21) in the sample; and,
(c) determining if the level of TCTPp21 is greater than the level of TCTPp21 in a normal sample. The method is useful in cancer in general and including the following cancers: breast cancer, lung, ovarian cancer, cervical cancer, prostrate cancer and colon cancer. Preferred direct detection methods are described herein but include electrophoresis procedures and immunoassays such as Western blot assays, enzyme-linked immunoassays (ELISA), fluorescent immunoassays, radioimmunoassays and the like. Indirect methods include using a nucleic acid hybridization assay; such assays would include Northern assay format and the polymerase chain reaction technology to detect specific mRNA.

The same methods apply to non-cancerous and actively growing cells.

The invention further provides for kits which detect growing cells. These kits comprise at a minimum a container containing a TCTPp21 binding analyte for detecting the level

of TCTPp21 in a tissue sample and a container containing a known amount of a TCTPp21 specific ligand. The binding analytes would include antibodies specific to TCTPp21 and the TCTPp21 specific ligands ar typically TCTPp21 or fragments thereof or nucleic acids which bind to mRNA encoding TCTPp21.

Abbreviations and Definitions

Abbreviations used:

PDA: piperazine-diacrylamide; PVDF: polyvinylidene difluoride; SDS: sodium dodecyl sulfate; 2D-PAGE: two-dimensional polyacrylamide gel electrophoresis; CAPG: carrier ampholyte pH gradients; IPG: immobilized pH gradients; HPLC: high pressure liquid chromatography.

Definitions:

"Cancerous conditions" refers to a disease defined by unregulated cell growth in a mammalian host.

"TCTPp21 binding analyte" refers to a reagent which binds specifically to TCTPp21 or to a nucleic acid encoding TCTPp21. Such reagents would include an antibody, either a polyclonal mixture or monoclonal antibody, which binds specifically to TCTPp21 or to a cDNA which will hybridize to nucleic acid encoding TCTPp21 or portions thereof.

"TCTPp21 specific ligand" refers to a reagent which binds to a TCTPp21 binding analyte and would include TCTPp21 either native or recombinantly produced and nucleic acid encoding TCTPp21 or portions thereof.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1:

Figure 1 shows a silver stained human liver protein 2D-PAGE picture obtained with non linear immobilized pH gradient (IPG) from 3.5 to 10 for the first dimensional separation.

Figure 2:

Figure 2 shows a computer match between silver stained 2D-PAGE images of liver (figure 1), plasma, red blood cell, rectal adenocarcinoma samples and an amido black stained PVDF membrane image of liver sample (figur 5). This figure was done using allspots, allareas, viewmod, modified automatch, showpairs, metal, gelsharper and showgroups programs of the Melanie/Elsie computer system [references Nos. 68, 71]. The vectors link a few matched spots between the liver "master" picture, the PVDF membrane and the other type of samples. TCTP means translationally controlled tumor protein.

Figure 3:

Figure 3 shows an enlargement of the acidic and lower molecular weight area of figure 1. "U" means unknown sequence in Swiss-Prot database. The numbers represent sequenced peptides or peptides which were attempted to be sequenced. some polypeptides could not be N-terminally microsequenced either because of too low protein concentration or because of N-terminal blockage. SRBP means serum retinol binding protein.

Figure 4:

Figure 4 shows an enlargement of the basic area of figure 1. "U" means unknown sequence in Swiss-Prot database. The numbers again identify spots which were the subject of peptide sequencing.

Figure 5:

Figure 5 shows one of the 5 to 9 amidoblack stained PVDF membranes which have been used to microsequenced 102 spots. The numbers provide a reference to figures 2-3. TCTPp21 is found at number 53.

The same membrane picture is also shown on figure 2.

DETAILED DESCRIPTION

This invention provides novel methods for detecting cell growth in humans and other mammals. The method relies upon the disc very that a previously known gene produces a protein which is specific for dividing cells. As such the presence and quantity of the protein is useful as a marker for detecting normal growth and cancerous growths.

TCTPp21 DNA and mRNA has been studied and cloned in plants (75), mouse erythroleukemia (73), mouse ascitic tumor (72) and human mammary carcinoma (74). The cDNA sequence coding for TCTP was obtained by screening a cDNA library constructed in lambda gt10 from human mammary carcinoma. The sequence is provided in Seq. ID No. 1.

Probes derived from cDNA of the translationally controlled, growth-related mouse tumor protein p23, were used to detect the clone. TCTPp21 has no known function, but its high degree of homology from plants to man underlines its probable crucial role in cell function. Previous studies showed that mouse TCTP protein concentration increased with mouse tumor growth and that its production was translationally controlled and mouse serum dependent.

A. Obtaining Clinical Samples

TCTPp21 is elevated in growing cells. The cells can be collected by conventional surgical means. A surgeon will remove tissue samples or fluid samples from a patient. The samples are suspected of containing TCTPp21 in elevated levels. Said elevated levels are indicative of active cell growth which in the case of a cancer is unregulated. Where cell death is suspected due to chemotherapy or to a natural process intrinsic to active growth of cells, TCTPp21 is expected to be present in the lymph and blood fluids of the patient.

Where tissue samples are obtained, the cells are typically lysed, then diluted in a physiologically compatible buffer such as phosphate or HEPES at a pH of between 6.5 and 7.5. The cells are lysed using a variety

of standard means which include pressure, shearing, or osmotic pressure. The whole lysate can be used in immunoassays or is optionally semi-purified to remove unwanted cellular material.

B. Recombinant Expression of TCTPp21.

Complementary DNA encoding TCTPp21 has been previously described by Gross, B.,Gaestel, M.,Bohm, H. & Bielka, H. (1989), *cDNA sequence coding for a translationally controlled human tumor protein,* Nucleic Acids Res, 17(20), 8367. Using conventional recombinant technology one can obtain unlimited quantities of TCTPp21. A general review of this technology can be found in Sambrook, et al., *Molecular Cloning - A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 1989. In brief the cDNA described by Gross *et al.* is recombined with an expression vector and transformed into a host cells competent to express heterologous genes. The product is then isolated and purified from the recombinant host. Such cells would include insect cells, bacterial cells and mammalian cells. The choice of vector and cells are not critical and are determined by convenience and economy.

C. Direct Methods for Detecting TCTPp21.

There are two basic methods for directly measuring the presence of TCTPp21. Both methods are routine and conventional techniques. No attempt will be made to exhaustively identify all the variations possible. The two methods are electrophoretic techniques whereby the TCTPp21 is isolated from other products by its molecular size and charge and immunoassays whereby the TCTPp21 is detected by its ability to be recognized by TCTPp21 specific antibodies.

Electrophoretic procedures are well known and generally described in the book, Electrophoresis of Proteins in polyacrylamide gels and starch gel, Eds. Work and Work, Elsevier Science Publ. 1983. Such methods include separation of TCTPp21 from other proteins based upon its size or relative charge. The separated protein

is then detected using a variety of techniques which include colorimetric staining, radiolabels, fluorecent tagging and the like. Specific electrophoresis methods include SDS-Polyacrylamide gel electrophoresis, capillary electrophoresis, electrophoresis under nondenatured and denatured conditions.

Immunoassays are also well known and reviewed in Harlow and Lane, *Antibodies: A Laboratory Manual,* Cold Spring Harbor Pubs., N.Y. (1988). This technique relies upon the production of antibodies specific to TCTPp21. The antibodies can be either polyclonal or monoclonal. The antigens are preferably intact TCTPp21 or peptides representing the most hydrophyllic regions.

The immunoassay formats include Western blots, ELISA, fluorescent and radioimmunoassays and the like. In brief samples suspected of containing TCTPp21 are obtained and contacted with antibodies under controlled conditions. The binding of the antibodies is then detected in a variety of standard ways.

Other direct methods for detecting TCTPp21 would include high performance liquid chromatography and reverse phase liquid chromatography.

D. Indirect Methods for Detecting TCTPp21.

TCTPp21 can be detected using indirect means whereby levels of mRNA are measured. Such procedures rely upon the hybridization of complementary nucleic acids. A general review of this technology can be found in Nucleic Acid Hybridization a Practical Approach, Eds. B.D. Hames and S.J. Higgins, IRL Press, Washington D.C. USA, 1989. In general, the cells in a sample are lysed and the mRNA of a tissue sample is isolated using a conventional technique such as with a polyoligo dT column. The isolated mRNA is then assayed using probes which could include the entire cDNA sequence for TCTPp21 mRNA as provided in Seq. ID No. 1 or specific fragments thereof. Other operable methods would include isolation of whole nucleic acid and detection of mRNA in the mixture.

A basic technique for detecting mRNA is the Northern blotting technique where mRNA is separated according to size in an electrophoretic matrix. The mRNA is then transferred to a solid support and labelled cDNA probes are used to detect the presence of specific mRNA.

A second means to identify specific MRNA in a cell lysate involves the use of the polymerase chain reaction. A general review of this technology is found in PCR Protocols A Guide to Methods and Applications, Eds., Innis, Gelfand, Sninsky and White, Academic Press, Inc. 1990. Chapter 8 entitled "Competitive PCR for Quantitation of mRNA" describes this technology in detail.

E. Kits

This invention further provides for kits. Such kits would include materials and instructions for detecting TCTPp21 either directly or indirectly as described above. The kits would contain reagents such as recombinant TCTPp21, TCTPp21 specific antibodies and miscellaneous reagents for controls, PCR primers and TCTPp21 specific nucleic acid probes, detection reagents such as enzyme/substrate combinations, fluorescent labels, solid supports for antibodies or nucleic acids and the like. The specific combination of reagents are determined by the assay being presented by the kit.

Examples

The examples provided by way of illustration only and not by way of limitation. Those of skill will readily recognize a variety of noncritical parameters which could be changed or modified to yield essentially similar results.

Example 1. The use of 2D electrophoresis mapping to identify TCTPp21 as a new cancer marker.

The following materials and methods were used to determine that the expression levels of TCTPp21 were elevated in growing cells:

Acrylamide, piperazine diacrylamide, tetramethyl-ethylenediamine, ammonium persulfate, glycine, sodium

dodecylsulfate were from Bio-Rad (Glattbrugg, Switzerland); PVDF membranes were from Millipore (Bedford, MA,USA) or Bio-Rad; tris(hydroxymethyl)-aminomethane (hydrochl ride), Nonidet P-40®, chloramidopropyl-dimethyl-hydroxypropane-sulfonate were from Sigma (Deisenhofen, Germany); citric acid, urea and dithiothreitol were purchased from Merck (Darmstadt, Germany) and Resolytes™ 4-8 from Chemie Brunschwig (Basel, Switzerland); Immobiline® were from Pharmacia-LKB (Uppsala, Sweden).

In the early course of this work, 2D-PAGE using carrier ampholytes in the first dimension was performed as described previously [44, 45]. The isoelectrophoretic separation was performed in a model 175 chamber with a model 3000/300xi power supply from Bio-Rad. For SDS-PAGE, the Protean II™ chamber and casting chamber (160 x 200 x 1.5 mm gels) were used. The power supply (700V, 1.6 A) was from Hewlett-Packard (Palo Alto, CA). The gradient pourer was the Bio-Rad model 395.

More recently, the isoelectrophoretic separation (immobilized pH gradients) was performed in a Multiphore™ apparatus with a 5000 V power supply from Pharmacia/LKB (Broma,Sweden). Preparative isoelectrofocusing was also done using narrow immobilized pH gradients and the equipment mentioned above. In later experiments, the gradient pourer was the computerized gradient pourer Angelique™ (LargeScaleBiology, Rockville, MD). N-terminal sequence determinations were performed using either a model 473A or 477A microsequencer (Applied Biosystems, Foster City, CA) equipped with Problott™ reaction cartridges.

Plasma and red blood cell sample collection and preparation were done according to previous publications [44, 46-48]. The liver and other biopsy samples were collected in the operating room at the time of surgery and immediately frozen in liquid nitrogen. They were prepared as follows.

Five frozen slices (20μm x 5 mm x 10 mm) of liver biopsy were mixed in an Eppendorf® tube with 300 μL of a

solution containing 8 M Urea, 4 % w/v CHAPS, 40 mM Tris, 10 mM DTE and a trace of Bromophenol Blue. Sixty μL of the final diluted liver sample were loaded on each IPG gel strip.

For micropreparative gel sample load the sample were treated as follows. Twenty frozen slices (20 μm x 5 mm x 10 mm) of liver biopsy were placed in an Eppendorf® tube with 400 μL of a solution containing 8 M Urea, 4 % w/v CHAPS, 40 mM Tris, 10 mM DTE and a trace of Bromophenol Blue. Ninety μL of the final diluted liver sample were loaded on each IPG gel strip.

In addition, rectal cancer, lung cancer, normal liver and brain tissue samples were prepared according to the above protocol.

A. Two-dimensional polyacrylamide gel electrophoresis

Two-dimensional gel electrophoresis was performed essentially as described previously for carrier ampholyte pH gradient separation **[44, 45]** and as described by Goerg et al. [49] for immobilized pH gradients. Running conditions for the first dimensional separation were as follows: voltage was linearly increased from 300 V to 3500 V during the first 3 hours; the strips were allowed to run at 3500 V for 3 additional hours and the voltage was then increased to 5000 V to give a total of 100 KVh for analytical gels and 450 KVh in a 4 days run for micropreparative gels. After the second dimension, the gels were stained with silver **[44, 45, 50, 51]**. When 2D-PAGE was followed by electroblotting and microsequencing analysis (see below), the amount of sample loaded in the first dimension was multiplied by 5 as mentioned above.

B. Electroblotting, staining and microsequencing procedures

Electroblotting onto PVDF membranes was done essentially according to Towbin et al. **[52]** and Matsudaira **[53]**. Membranes were stained with amidoblack, destained with water and dried. Spots of interest were excised, dried under

nitrogen and kept in Eppendorf® tubes at -20°C until microsequencing was performed [53-57].

Routinely, ten to twelve Edman degradation cycles (see section 2.2) w re performed for each spot. A search in the Swiss-Prot database [58] was made to establish identity or homology to already known proteins.

Results

C. Carrier ampholytes versus immobilized pH gradients

In the past, human liver protein maps, obtained using carrier ampholyte pH gradients for the first dimensional separation, could not be compared to plasma, red blood cell or rectal cancer tissue maps. The cathodic drift, seen with carrier ampholyte pH gradients, altered the focusing of basic polypeptides too much. Furthermore, proteins as well as ions present in the sample influenced the pH gradient. Consequently, the pH gradient varied from sample to sample. Immobilized pH gradients circumvented this problem and provided much more reproducible patterns. It was thus possible to localize proteins on the immobilized pH gradient 2D-PAGE pictures by direct comparison of different sample separations (liver, rectal adenocarcinoma tissue, red blood cell and plasma) (see figures 2-4).

D. Previously identified proteins

In plasma/serum maps, more than 350 spots representing more than 40 proteins have been identified so far [25, 26, 46, 47, 59-63]. In red blood cell maps, 12 polypeptides have been identified so far [27, 48]. Figures 2 and 3 show the localisation of several plasma and red blood cell polypeptide chains on the human liver protein map (enlargements of the silver stained immobilized pH gradient 2D-PAGE image of figure 1). The identification was done by overlapping liver, red blood cell and plasma protein maps. These proteins could be either trapped in the liver blood vessels or localized in the bile ducts, portal area or hepatocytes.

E. Newly identified polypeptide sp ts

A group of more than 3000 spots, probably representing more than 2500 polypeptide chains, are readily detected by the Melanie computer system **[64-68]** on silver stained 2D-PAGE image of liver biopsy samples. In order to build our knowledge of the human liver protein map (and other tissues as well), to provide further information in disease associated protein pattern changes and to discover new liver proteins, we have attempted to N-terminally microsequence 102 spots transblotted onto PVDF membranes. A maximum of 9 2D-PAGE were run for any unique sequence determination. We did not obtain any sequencing signal for 43% of the spots either because of too low a protein concentration or because of N-terminal blockage. In one case, a double sequencing signal was obtained, not providing any possible interpretation (spot number 50, figures 4 & 5). Two spots (2%) gave double sequencing signals providing the identification of two polypeptides (spot numbers 71 and 98, figures 4 & 5). For 11 % of the spots, the sequence was unequivocal, but no matches could be found in the Swiss-Prot database. Those were considered as unknown proteins. 44% of the spots were positively identified as known proteins or derived fragments.

Figure 5 shows an amidoblack stained PVDF membrane picture. The red arrows highlight the spots wich have been successfully N-terminally microsequenced. The blue arrows highlight the spots wich have not been successfully N-terminally microsequenced. The numbers provide references to table 1. Spot number 53 was found to be the translationally controlled tumor protein (spot number 53, figures 2,3 & 5).

Based on this discovery, we did further study.
The liver piece used in this study was obtained from a patient who suffered a neuroendocrine tumor. The tumor originated in the pancreas and metastasized in the liver. The patient underwent successfully a right hemi-hepatectomy for tumor mass reduction. We microsequenced a 21 Kd acidic spot which was found to be the translationally controlled tumor protein (spot number 53, figures 2,3 & 5). This

protein spot was then found also on rectal adenocarcinoma biopsy (figure 2), on lung adenocarcinoma and on hepatocarcinoma derived cell line patterns, the latter having a pattern very similar to liver biopsies. The TCTP spot was not found in normal liver and brain maps.

Example 2. An immunoassay to detect cancer in a human patient.

A patient suspected of hosting cancerous liver cells is biopsied using conventional surgical techniques. The liver tissue is homogenized and the cell lysate is diluted with a physiological buffer. A plastic ELISA titration plate having a first section of holes previously treated with TCTPp21 specific antibodies is pre-prepared. A second section of the plate provides control antibodies which do not bind to TCTPp21. A portion of both sections is contacted with various dilutions of the sample and with a control solution containing TCTPp21. The plates are incubated and then washed. The bound TCTPp21 is then detected using TCTPp21 specific antibodies bearing a fluorescent or enzyme label.

Example 3. A nucleic acid hybridization assay to det ct cancer in a human patient.

The sample from the patient described in example 2 is homogenized and the total mRNA extracted using conventional mRNA purification technology which takes advantage of the unique polyA tails of mRNA. The mRNA is then assayed using the Northern blotting techniques taught in Sambrook using a cDNA probe derived from the sequence provided herein encoding TCTPp21.

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 781 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: c-DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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 61  CTAGCGCCGTCGTCGTCTCCCTTCAGTCGCCATCATGATTATCTACCGGGACCTCATCA
                                          M  I  I  Y  R  D  L  I
121  CCACGATGAGATGTTCTCCGACATCTACAAGATCCGGGAGATCGCGGACGGGTTGTGCC
      H  D  E  M  F  S  D  I  Y  K  I  R  E  I  A  D  G  L  C
181  GGAGGTGGAGGGGAAGATGGTCAGTAGGACAGAAGGTAACATTGATGACTCGCTCATTG
      E  V  E  G  K  M  V  S  R  T  E  G  N  I  D  D  S  L  I
241  TGGAAATGCCTCCGCTGAAGGCCCCGAGGGCGAAGGTACCGAAAGCACAGTAATCACTG
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361  GAAGTACATCAAAGATTACATGAAATCAATCAAAGGGAAACTTGAAGAACAGAGACCAG
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421  AAGAGTAAAACCTTTTATGACAGGGGCTGCAGAACAAATCAAGCACATCCTTGCTAATT
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      D  Y  R  E  D  G  V  T  P  Y  M  I  F  F  K  D  G  L  E
601  GGAAAAATGTTAACAAATGTGGCAATTATTTTGGATCTATCACCTGTCATCATAACTGG
      E  K  C
661  TTCTGCTTGTCATCCACACAACACCAGGACTTAAGACAAATGGGACTGATGTCATCTTG

721  GCTCTTCATTTATTTTGACTGTGATTTATTTGGAGTGGAGGCATTGTTTTTAAGAAAAA

781  ATGTCATGTAGGTTGTCTAAAAATAAAATGCATTTAAACTCATTTGAGAGAAAAAAAA
```

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Hochstrasser, Denis F.
Hughes, Graham J.
Appel, Ron D.

(ii) TITLE OF INVENTION: A Method for Detecting Growing Cells Using Translationally Controlled Tumor Protein p21

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: KATZAROV S.A.
(B) STREET: 19, rue des Epinettes
(C) CITY: Geneva
(D) STATE: GE
(E) COUNTRY: Switzerland
(F) ZIP: 1227

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/990,302
(B) FILING DATE: 02-DEC-1992
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Reverdin, André

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (+4122) 342 66 30
(B) TELEFAX: (+4122) 342 66 15

```
(2) INFORMATION FOR SEQ ID NO:1:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 838 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
          (A) NAME/KEY: CDS
          (B) LOCATION: 95..610

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCCCCCCGAG CGCCGCTCCG GCTGCACCGC GCTCGCTCCG AGTTTCAGGC TCGTGCTAAG
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CTAGCGCCGT CGTCGTCTCC CTTCAGTCGC CATC ATG ATT ATC TAC CGG GAC
   112
                                      Met Ile Ile Tyr Arg Asp
                                        1               5

CTC ATC AGC CAC GAT GAG ATG TTC TCC GAC ATC TAC AAG ATC CGG GAG
   160
Leu Ile Ser His Asp Glu Met Phe Ser Asp Ile Tyr Lys Ile Arg Glu
             10                  15                  20

ATC GCG GAC GGG TTG TGC CTG GAG GTG GAG GGG AAG ATG GTC AGT AGG
   208
Ile Ala Asp Gly Leu Cys Leu Glu Val Glu Gly Lys Met Val Ser Arg
         25                  30                  35

ACA GAA GGT AAC ATT GAT GAC TCG CTC ATT GGT GGA AAT GCC TCC GCT
   256
Thr Glu Gly Asn Ile Asp Asp Ser Leu Ile Gly Gly Asn Ala Ser Ala
     40                  45                  50

GAA GGC CCC GAG GGC GAA GGT ACC GAA AGC ACA GTA ATC ACT GGT GTC
   304
Glu Gly Pro Glu Gly Glu Gly Thr Glu Ser Thr Val Ile Thr Gly Val
 55                  60                  65                  70

GAT ATT GTC ATG AAC CAT CAC CTG CAG GAA ACA AGT TTC ACA AAA GAA
   352
Asp Ile Val Met Asn His His Leu Gln Glu Thr Ser Phe Thr Lys Glu
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GCC TAC AAG AAG TAC ATC AAA GAT TAC ATG AAA TCA ATC AAA GGG AAA
   400
Ala Tyr Lys Lys Tyr Ile Lys Asp Tyr Met Lys Ser Ile Lys Gly Lys
             90                  95                 100

CTT GAA GAA CAG AGA CCA GAA AGA GTA AAA CCT TTT ATG ACA GGG GCT
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Leu Glu Glu Gln Arg Pro Glu Arg Val Lys Pro Phe Met Thr Gly Ala
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 172 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Ile Ile Tyr Arg Asp Leu Ile Ser His Asp Glu Met Phe Ser Asp
 1               5                  10                  15

Ile Tyr Lys Ile Arg Glu Ile Ala Asp Gly Leu Cys Leu Glu Val Glu
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Gly Lys Met Val Ser Arg Thr Glu Gly Asn Ile Asp Asp Ser Leu Ile
        35                  40                  45

Gly Gly Asn Ala Ser Ala Glu Gly Pro Glu Gly Glu Gly Thr Glu Ser
    50                  55                  60

Thr Val Ile Thr Gly Val Asp Ile Val Met Asn His His Leu Gln Glu
65                  70                  75                  80

Thr Ser Phe Thr Lys Glu Ala Tyr Lys Lys Tyr Ile Lys Asp Tyr Met
                85                  90                  95

Lys Ser Ile Lys Gly Lys Leu Glu Glu Gln Arg Pro Glu Arg Val Lys
            100                 105                 110

Pro Phe Met Thr Gly Ala Ala Glu Gln Ile Lys His Ile Leu Ala Asn
        115                 120                 125

Phe Lys Asn Tyr Gln Phe Phe Ile Gly Glu Asn Met Asn Pro Asp Gly
    130                 135                 140

Met Val Ala Leu Leu Asp Tyr Arg Glu Asp Gly Val Thr Pro Tyr Met
145                 150                 155                 160

Ile Phe Phe Lys Asp Gly Leu Glu Met Glu Lys Cys
                165                 170
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GCA GAA CAA ATC AAG CAC ATC CTT GCT AAT TTC AAA AAC TAC CAG TTC
    496
Ala Glu Gln Ile Lys His Ile Leu Ala Asn Phe Lys Asn Tyr Gln Phe
     120                125                 130

TTT ATT GGT GAA AAC ATG AAT CCA GAT GGC ATG GTT GCT CTA TTG GAC
    544
Phe Ile Gly Glu Asn Met Asn Pro Asp Gly Met Val Ala Leu Leu Asp
135                 140                 145                 150

TAC CGT GAG GAT GGT GTG ACC CCA TAT ATG ATT TTC TTT AAG GAT GGT
    592
Tyr Arg Glu Asp Gly Val Thr Pro Tyr Met Ile Phe Phe Lys Asp Gly
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TTA GAA ATG GAA AAA TGT TAACAAATGT GGCAATTATT TTGGATCTAT
    640
Leu Glu Met Glu Lys Cys
            170

CACCTGTCAT CATAACTGGC TTCTGCTTGT CATCCACACA ACACCAGGAC TTAAGACAAA
    700

TGGGACTGAT GTCATCTTGA GCTCTTCATT TATTTTGACT GTGATTTATT TGGAGTGGAG
    760

GCATTGTTTT TAAGAAAAAC ATGTCATGTA GGTTGTCTAA AAATAAAATG CATTTAAACT
    820

CATTTGAGAG AAAAAAAA
    838
```

WHAT IS CLAIMED IS:

1. A method of detecting a cancerous condition in a human comprising:

(a) removing a clinical sample from the human;

(b) detecting the level of translationally controlled tumor protein p21 (TCTPp21) in the sample; and,

(c) determining if the level of TCTPp21 is greater than the level of TCTPp21 in a normal sample.

2. A method of claim 1 wherein the cancerous condition is selected from the group of cancers consisting of: breast cancer, ovarian cancer, cervical cancer, prostrate cancer and colon cancer.

3. A method of claim 1 wherein the level of TCTPp21 is detected using an immunoassay.

4. A method of claim 3 wherein the immunoassay is an ELISA format.

5. A method of claim 1 wherein the level of TCTPp21 is indirectly detected using a nucleic acid hybridization assay.

6. A method of claim 1 where the TCTPp21 is detected using electrophoresis.

7. A method of detecting growing cells in a human comprising:

(a) removing a clinical sample from the human;

(b) detecting the level of translationally controlled tumor protein p21 (TCTPp21) in the sample; and,

(c) determining if the level of TCTPp21 is greater than the level of TCTPp21 in a non-growing sample.

8. A method of claim 7 wherein the level of TCTPp21 is detected using an immunoassay.

9. A method of claim 8 wherein the immunoassay is an ELISA format.

10. A method of claim 7 wherein the level of TCTPp21 is indirectly de
tected using a nucleic acid hybridization assay.

11. A method of claim 7 where the TCTPp21 is detected using electrophoresis.

12. A kit for detecting growing cells which comprises: a container containing a TCTPp21 binding analyte for detecting the level of TCTPp21 in a tissue sample and a container containing a known amount of a TCTPp21 specific ligand.

13. A kit of claim 12 wherein the binding analyte is an antibody specific for TCTPp21.

14. A kit of claim 12 wherein the binding analyte is a nucleic acid which hybridizes to a mRNA encoding TCTPp21.

15. A kit of claim 12 wherein the TCTPp21 specific ligand is TCTPp21.

16. A kit of claim 12 wherein the TCTPp21 specific ligand is a nucleic acid complementary to the human cDNA encoding TCTPp21.

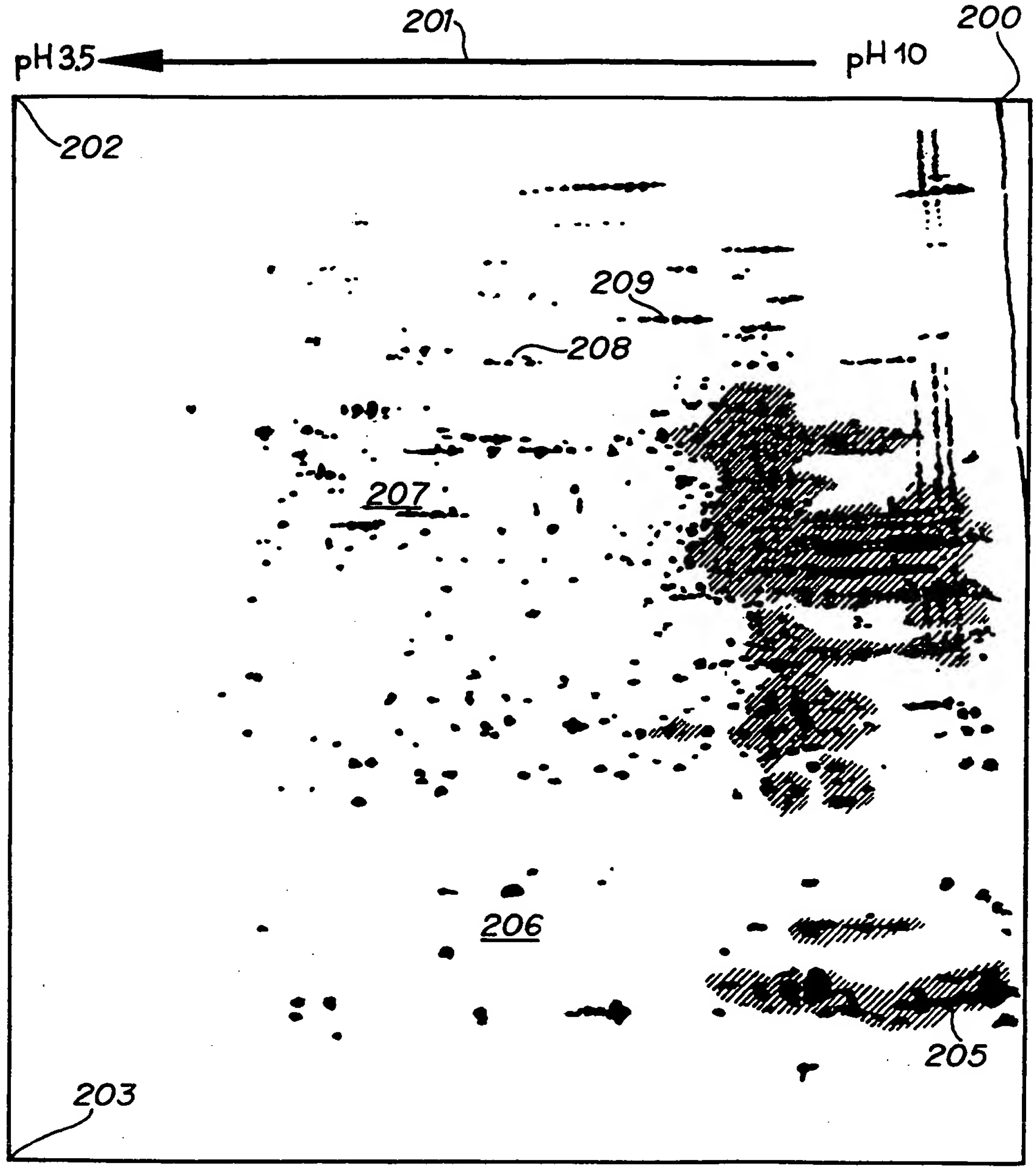
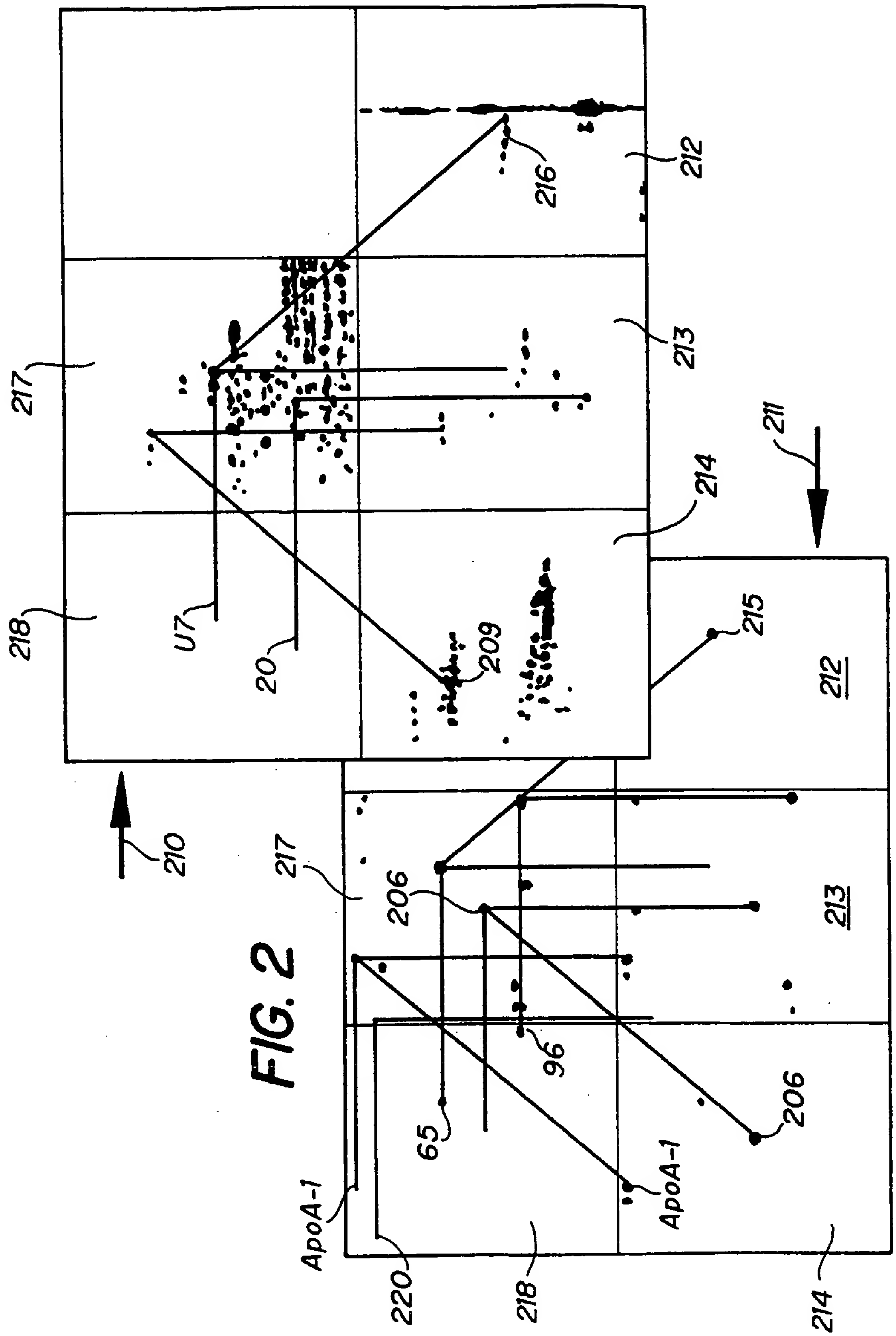


FIG. 1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U7
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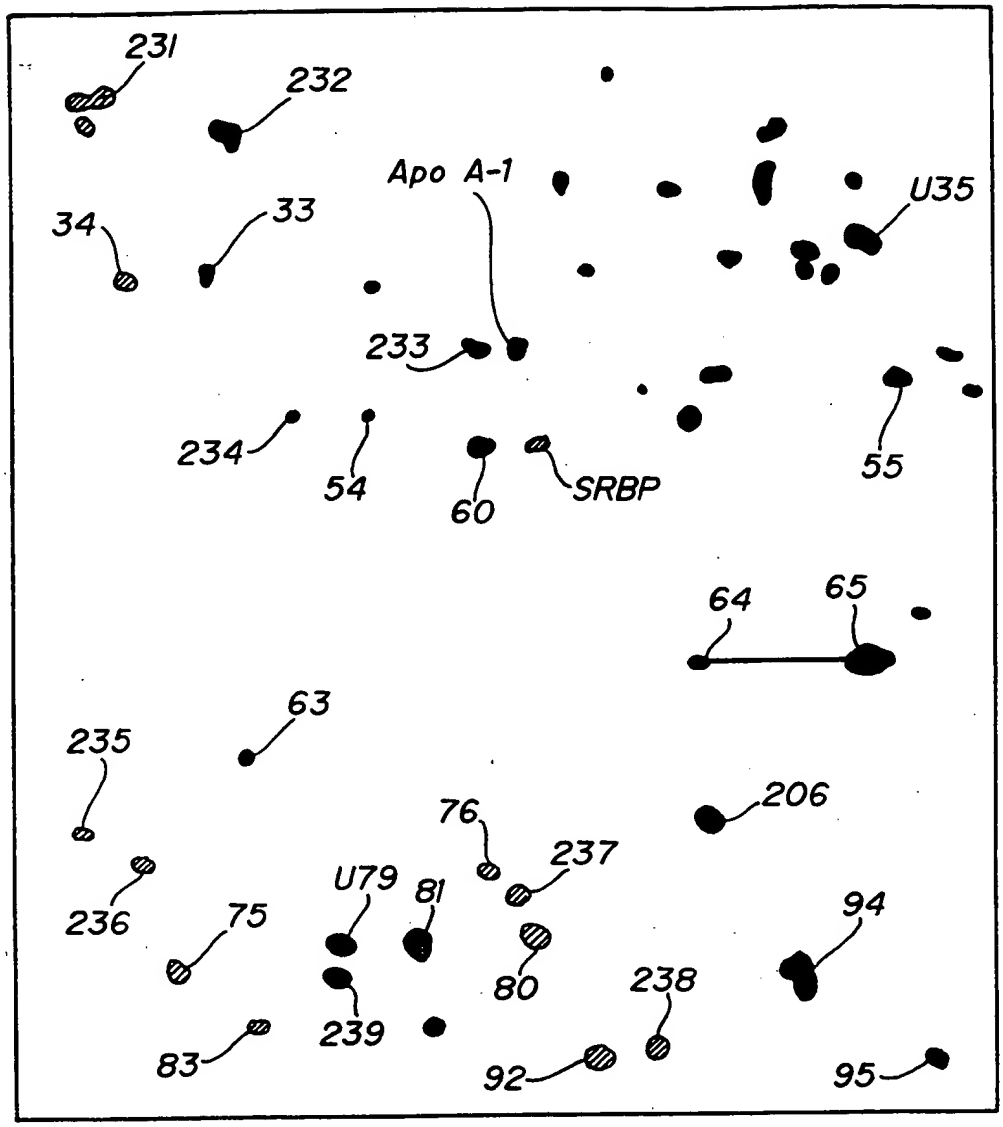
206
ApoA-1
ApoA-1
220
218
214

FIG. 2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SRBP
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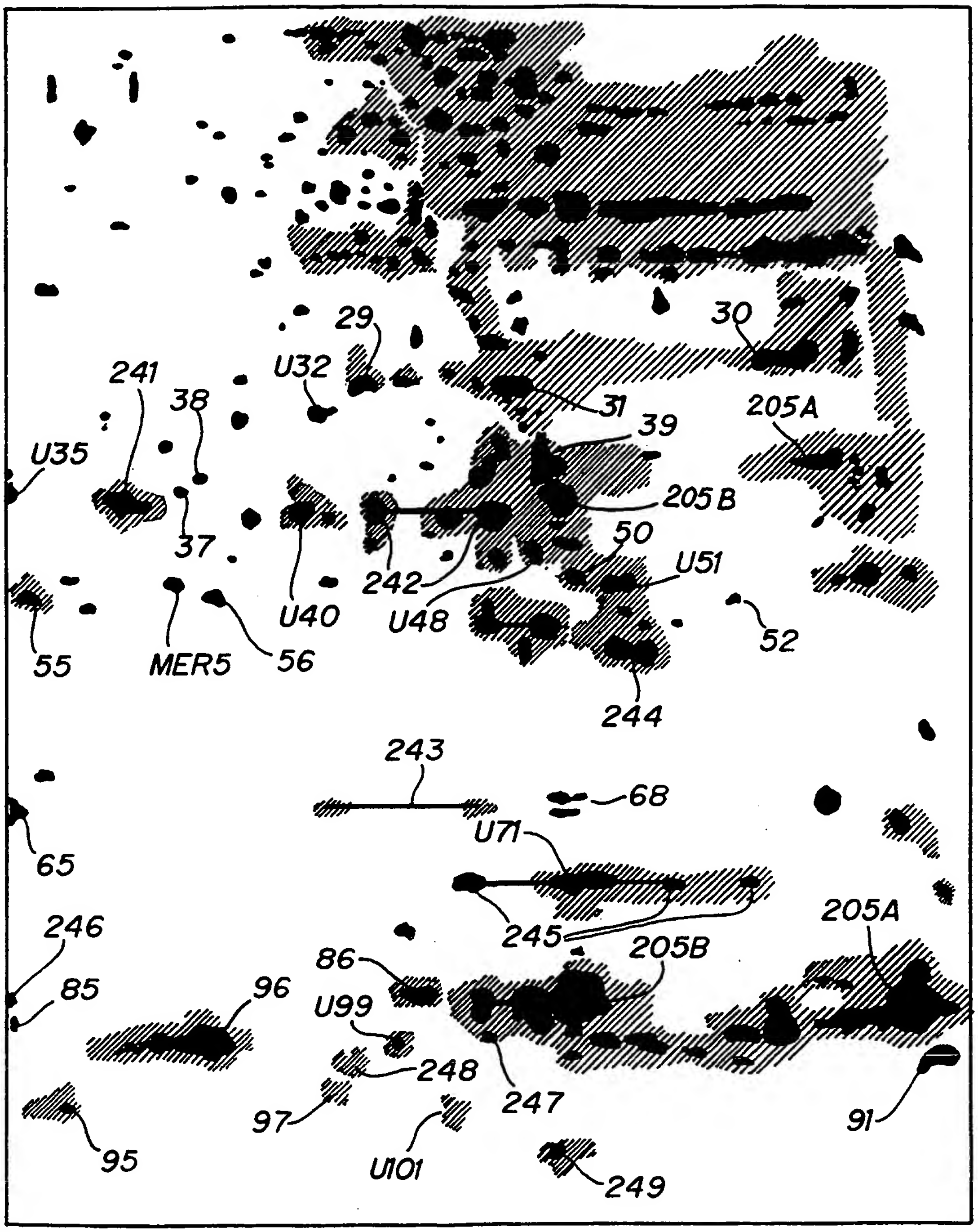
FIG. 3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52
244
243
68
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U99
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247
97
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U101
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FIG. 4

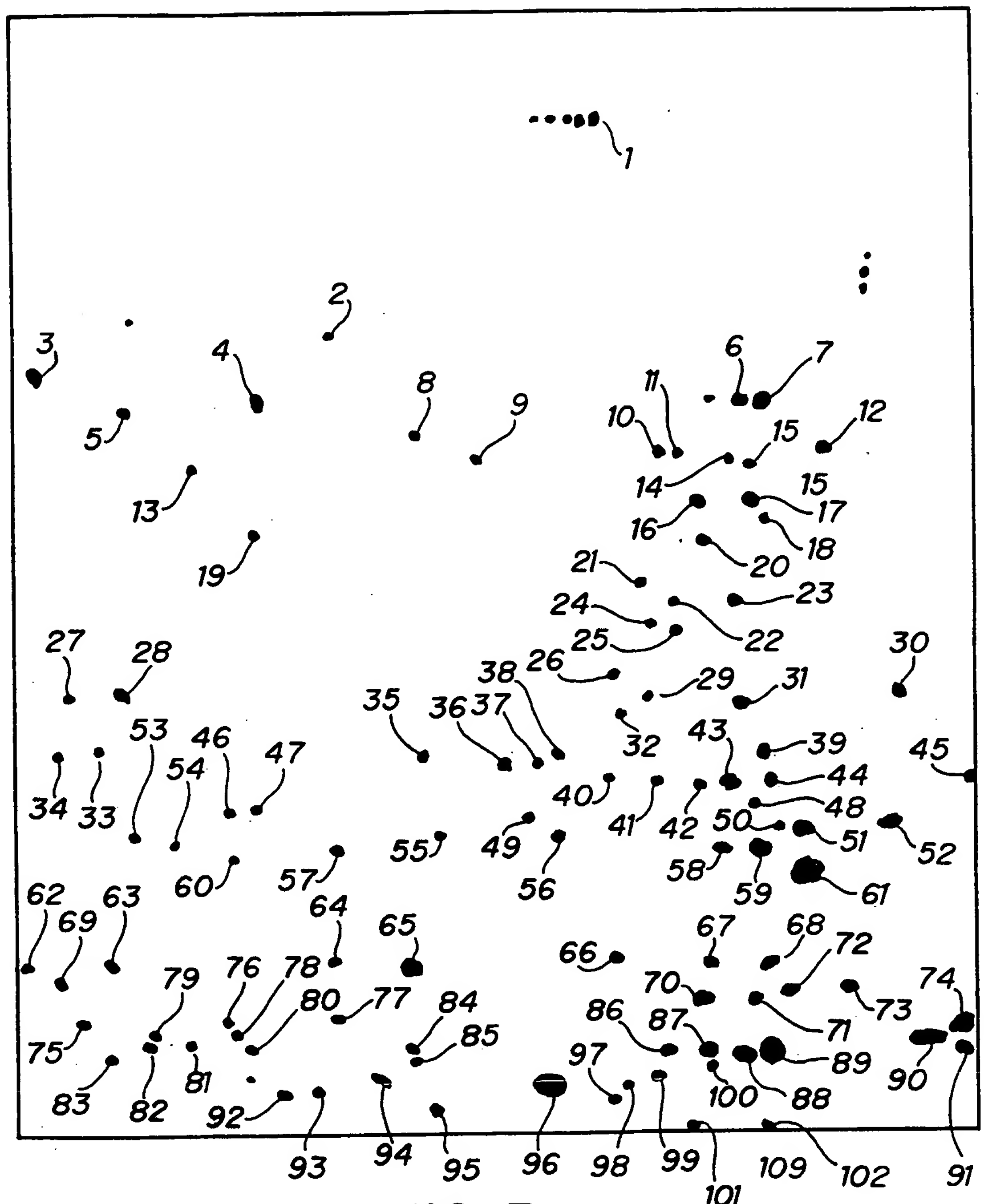
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FIG. 5